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Current Approaches to the Determination of Feed Intake and Digestibility in Ruminant Animals – A Review

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ABSTRACT
The objective of this paper is to review the current approaches for determining feed intake and digestibility in ruminant animals**.** Plant species or parts can differ markedly in nutritive value. The botanical composition of consumed herbage can have a profound effect on the provision of nutrients to the animal. To evaluate the preferred diet intake of grazing animals, it is necessary to spatially separate the forages being evaluated to eliminate the constraints that occur within mixed forages. In livestock production context, this is especially relevant for mixed grass and legume forages, because the consumption of legume will usually result in better animal performance. For rangeland cattle, the higher consumption of some plant species rather than others not only has nutritional effects on the animal but can also have an important influence on the species composition of the plant biomass, with consequences for ecological sustainability. Initially, quantitative estimation of the different ingested plant fractions was commonly practiced. It however, during the last four decades, many empirical models have been developed to estimate forage intake at pasture. Most of these models were derived from dairy cows experimental data, which were often specific to the relatively short range of grazing and experimental conditions used to develop them. Recent research approaches for segregating mixed swards however employ the N-alkanes profiles determination method which have been developed from different methodologies and have been used successfully to determine the proportion of different plant species in the diet. Furthermore, the oral administration of N-alkanes has been used in digestibility trials with domestic and wild ruminants as well as monogastric animals to measure feed intake and digestibility of the available herbage. Similarly, indigestible internal plant markers such as lignin and acid detergent fibres are being used to determine digestibility in ruminants. N-alkanes can be supplied to animals in different forms. Pellet feed made of paper strip embedded with synthetic N-alkanes as external markers has been used to estimate feed intake of sheep. Since evaluation of preferred forage will continue to be important in grazing lands development, researchers in regions trying to move from traditional extensive pastoral practices to some form of semi-sedentary production under managed fields may find these approaches very useful. As these novel approaches continue to evolve, it is expected that they will become simplified and cost effective and therefore find wider application in agriculture and diagnostic research.

Keywords: Forage, Feed Intake, Digestibility, N-alkane, Plant markers, Ruminants

INTRODUCTION

Forage is the most economical ruminant feed during the grazing season [1]. The establishment of mixed pasture systems by including legumes and forbs, however, has gained interest as many seek to increase biodiversity [2]. In such diverse landscapes, the ability to determine dry matter intake (DMI) and digestibility is a valuable area of study due to its impact on the nutritional status, productivity and health of animals. It also adds to our knowledge of ruminant foraging behavior and impact of seasons on biodiversity and the dynamics of the plant community [3, 4]. A considerable amount of research has been conducted using long chain saturated hydrocarbons (N-alkanes) as markers to estimate feed intake and digestibility [5, 6, 7]. N-alkanes are saturated, aliphatic hydrocarbons with length varying from 21 to 37 carbon atoms [8]. They are part of the cuticular wax of plant leaves and are usually part of the ether extract which are indigestible in nature. The oral administration of N-alkanes has been used in digestibility trials with domestic and wild ruminants as well as monogastric animals to measure feed digestibility [9, 10] and feed intake [11, 12].

N-alkanes can be supplied to animals in different forms. Pellet feed made of paper strip embedded with synthetic N-alkanes as external markers was used to estimate feed intake of sheep. Similarly, [11] fed sheep with N-alkanes (C_{28} and C_{32}) in the form of gelatin capsules of powdered cellulose, previously added with a known amount of N-alkane dissolved with N-hexane or N-heptane, to estimate forage intake. [13] developed a different method that consists of mixing N-alkanes with solvents and powdered cellulose, resulting in a homogenous suspension that, after being evaporated and dried, was inserted into gelatin capsules. [14] developed another technique in which particles of *Pennisetum clandestinum* (Hochst.) were mixed with N-alkanes suspended in a xanthan gum (0.4%) and infused into the rumen of sheep using either drench guns or disposable syringes. In the same vein, indigestible internal plant markers such as lignin and acid detergent fibres are also used to determine feed digestibility [15, 16, 17]. Grazing time alone cannot be used to determine DMI of grazing animals because intake rate also must be considered [18]. The objective of this review is to highlight the technologies used for determination of feed intake and digestibility in ruminant animals.

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Using Plant Wax Alkanes to Estimate Feed Intake and Digestibility

The history of the use of plant wax components in the study of ruminant nutrition was presented in detail by [5]. Although the use of long chain fatty acids of plant wax, (C_{29} -C_{32}), as digestibility markers was advocated. Alkanes were first examined as digestibility markers [19]. They demonstrated that in sheep, the fecal recovery of alkanes was incomplete and progressively declined as carbon-chain length decreased. This has now been confirmed in several studies reviewed in detail [5]. [20] established that negligible synthesis of alkanes occurred in the ruminant digestive tract, and although alkanes were predominantly associated with paniculate matter in digesta, incomplete fecal recovery was due to absorption from the small intestine. Corrections for incomplete fecal recovery would be required if the natural alkanes were to be used on their own as markers to estimate digestibility. [19] found that the fecal recovery of C_{35} alkane was 0.975 and [5] later reported a mean C_{35} recovery in sheep of 0.948, Â ± 0.0102.

Based on an assumed fecal recovery of 0.95, C_{35} alkane has now been used successfully as a digestibility marker in sheep, providing estimates of digestibility which were more accurate than either in vitro estimates or those derived using lignin as a marker [21]. The validity of digestibility estimates obtained from C_{35} levels in diet and feces and an assumed fecal recovery value depends upon the degree to which the recovery value can vary. Errors in digestibility may be greater in cattle in which the fecal recovery of alkanes appears to be lower and more erratic than in sheep [5]. A further disadvantage of the use of C_{35} alkane as a marker to estimate digestibility is the fact that many plants have C_{35} in very low concentrations. [22] developed a double alkane procedure for estimating intake. In this approach, animals are dosed with known quantities of even-chain alkane and intake is estimated from the daily dose rate and the dietary and fecal concentrations of the dosed, even-chain alkane and a natural, odd-chain alkane adjacent in chain length. Note that in the feces, it is only the ratio of the alkane concentrations which is important. From their literature survey, [5] reported that alkanes C_{32} and C_{33} had recoveries of 0.868, Â ± 0.01 75 and 0.872, Â ± 0.0125, respectively. Due to the similar recoveries of adjacent alkanes, comparison of known intakes with those estimated using adjacent alkane pairs show good agreement.

The response is linear and amounts to an error of 1.25% in estimated intake for every percentage unit difference in recovery between the alkane pair. As [23] and [24] have discussed, errors in the estimate of digestibility when intake is estimated from the Cr_2O_3 in- vitro procedure, result in large errors in the estimated intake, especially when digestibility is high. The oral administration of synthetic, even-chain alkanes to animals has most frequently been carried out using once- or twice-daily dosing with either pellets of alkane-impregnated shredded paper [11, 22] or gelatin capsules containing alkanes suspended on powdered cellulose [11, 13]. Procedures for preparing pellets and capsules, and for analyzing alkanes in diet and feces have been discussed elsewhere [22, 5, 25, 13, 26, 27]. The analytical method of choice involves heating the sample with 1 mol/L ethanolic KOH in a closed tube for up to 16 h, followed by extraction into N-heptane; after purification through silica gel, the hydrocarbon fraction is analyzed by capillary gas chromatography. As with dosed Cr2O3, it takes 5-7 days from the commencement of alkane dosing to reach equilibrium concentrations in feces [11, 22, 16]. Within-day variation in the fecal concentration of dosed alkane is small for sheep dosed once daily with alkane-impregnated paper or twice daily with alkane on powdered cellulose, but may be higher with cattle [5]. However, the ratio of the fecal concentrations of the alkane pair used in the calculation of dietary intake is less prone to temporal variation than are the absolute concentrations [16]. To reduce the labor required for daily or more frequent dosing of animals with alkanes, which can become prohibitive in large scale intake studies, an intra-ruminal alkane CRD has been developed and its performance in sheep has been evaluated [16]. Release rates of the alkanes in the CRD were shown to be constant and were within 1.5-4.0% of the nominal release rates. The coefficient of variation of release rate between animals was 4.07%, and the intake estimated using the CRD was almost identical to known herbage intake. As with the CRD developed to administer Cr_2O_3, the alkane CRD has advantages over the once- or twice-daily dosing, of substantially reducing disturbance to the animals and minimizing the likelihood of diurnal variation effects. In-door validation studies have thus shown that the alkane procedure for estimating dietary intake is reliable [22, 13].

However, absolute validation of the method with grazing or browsing animals is virtually impossible to achieve, because alternative methods with which to compare the technique may be no more reliable or possibly inferior. Factors which may possibly influence the reliability of the technique when used with grazing or browsing animals, such as within- and between-day variations in feeding pattern have not been extensively studied. The main precaution required in the use of the method is to ensure that the diet sample, in terms of its alkane concentrations, is representative of that consumed by the experimental animals. For uniform, sown pastures, this is relatively easy to achieve by hand-gathering or by collecting extrusa samples from fasting fistulate animals [28].

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Under conditions in which animals can feed on complex vegetation communities, it may be extremely difficult or impossible to obtain feed samples having alkane concentrations which are representative of those in the diets of individual animals.

In such situations, the characterization of the botanical composition of the diet would enable intake to be assessed using the alkane technique in which the alkane concentrations of individual dietary components will have to be determined. While the alkane marker technique has been shown to give accurate assessments of dietary intake, the method has a number of other advantages. Provided that the alkane composition of the analyzed vegetation sample is representative of the diet of individual animals, the method allows for between-animal variation in diet digestibility and thus provides estimates of individual intakes. In contrast to the Cr_2O_3 in- vitro procedure [29], it can thus be used in genetic studies of the differences between individual animals in intake, digestibility and food conversion efficiency. Concurrent dosing with an external marker such as Cr_2O_3 to determine the fecal output allows the estimation of diet digestibility. However, dosed C_{36} alkane has been shown to have a consistently high fecal recovery 0.947, Â ± 0.0139 [5] and could thus be used in place of Cr_2O_3. This has the advantage that fecal C_{36} concentrations can be determined in the same analysis as for the alkanes used for intake estimation. The method can accommodate the feeding of supplements to the animals, provided individual intakes of supplement are known or can be estimated by other techniques such as those discussed [21]. Supplements with negligible alkane contents can be ignored in the intake calculation. Alternatively, if the feed supplement has an alkane pattern very different from that of the basal diet, the approach for measuring the botanical composition of the diet can be adopted.

This method can also be used to obtain individual intakes in group-housed animals. Individual intakes could also be determined in large-scale trials under feedlot conditions, or in commercial feedlots in which the conversion of feed to gain is a major determinant of profitability. Whereas, alternative methods of estimating intake such as the Cr_2O_3 in -vitro technique require separate analytical procedures for fecal output and digestibility estimation, the alkane technique requires a single analytical process. As will be discussed below, the method is readily extended to the simultaneous measurement of the botanical composition of the diet, so that the intake of individual species or plant parts can be quantified.

Intake Expression

Ways of expressing level of intake are numerous; forage dry or organic matter intake is expressed in weight unit per animal per day but the expression cannot be used to compare animal species or forages between them. For this purpose, intake may be expressed by kg of body weight raised to an exponent that can vary between 0.54 and 1.00 [30]. The choice of the exponent is a function of forage quality. With low quality forage, intake capacity of the animal appears more linked to the fill gut capacity and the rate of passage of forage. For such forages, the exponent is 1.00 and intake is expressed per kg of body weight or in percent of body weight [31]. Intake of good quality forage seems more controlled by physiological mechanisms. Intake of such forage is usually expressed per kg of metabolic weight (body weight raised to 0.75). The assumption is that intake is linked to energy requirements that are proportional to 0.75 power of body weight [32]. Nevertheless, a recent study by [33] has shown that, to compare intake level across forages and animal species, the best unit remains the dry matter intake in percent of body weight (DMI % BW). On this basis, relations between intake and passage rate of particles through the rumen or the energy digestibility appear independent of animal species. This is not the case when intake is expressed per kg of metabolic weight.

Level of Intake at Grazing

A first observation is that intake level related to animal is highly variable and linked to the characteristics of the forage. According to the French references, the *ad libitum* intake of a reference grass (15% of crude protein, 77% of organic matter digestibility on a dry matter basis) is 75, 95 and 140 g of dry matter per kg of metabolic weight respectively for a standard sheep, standard cattle (heifers) and a standard lactating dairy cow. On this basis, it is possible to calculate the "Fill Unit" ("*unité d'encombrement*") of various forages [34]. However, huge intake level variability also occurs between breed and individual animals within a given breed [35, 36]. As an example, Dorper sheep are less selective grazers as they consume more shrubs and bushes and ingest a larger number of different plant species than Merinos sheep [37].

The way of expressing intake level also contributes to this variability. As described by [33], DMI, expressed in % of body weight, appears higher for small ruminants (sheep and goats) than for cattle (dairy or suckling cattle), but this is the reverse when intake is expressed in terms of metabolic weight. Compared to temperate forages, voluntary intake of tropical forages is often lower but not highly different (2.03 kg DM per % BW *vs* 1.95 kg DM per % BW respectively for temperate and tropical forages), as confirmed in the meta-analysis of [38]. Despite, on a chemical point of view, temperate forages contain often higher proteins and lesser fibers. The difference decreases when the crude protein contents of both forages are similar. To explain the few differences, [38] indicated that tropical forages are usually longer chewed than temperate ones leading to a more efficient reduction of forage particles size compensating their lower apparent nutritive value.

ISSN: 0975 - 4539
IJBSAT (2013), 5(3):15-25

Factors Affecting Intake Regulation

The control of intake is multi-factorial as shown by [39, 40]. It depends on plants characteristics in relation to the gut capacity, animal's requirements and nutrients concentration of forage, post-ingestive feedback of the intake and the learning process. It also depends on morphological characteristics of grazed plants, and on the environment such as climate, abundance and frequency of feed resources [41].

Role of the Ruminal Fill

The fill gut capacity, in relation to forage characteristics, is considered as a main factor of regulation of voluntary intake. Intake appears limited by the maximal volume that the digestive tract can hold [32, 42], even if herbivores modify the volume of their rumen to increase the transit rate of digesta when the quality of forage decreased [43, 44, 45]. This has been confirmed by the introduction of tennis balls, water filled bags, or artificial fibres into the rumen. The more bulky the ruminal ballast is, in volume or in weight, the more the intake decreases with or without digestibility modification [45]. In a recent study, [46] confirmed that, ruminal fill can affect grazing behavior in terms of bite mass, bite depth and bite area. In this way, it is the short-term intake that is affected by the ruminal fill. In link to the ruminal fill, forages dry mater content can influence the voluntary intake. If dry matter of forage is lower than 20%, as in young grazed grass, the volume of water in the rumen increases and has depressive effect on the intake level, this in spite of a high forage digestibility [47, 30].

The age of plant regrowth is also a factor of variation. When plant protein content decreases, the cell walls and tissue lignification increases, as a consequence, there would be an increase in forage retention time in the rumen, limiting voluntary intake [48, 41, 49]. According to [50], the daily herbage intake of lactating dairy cows decreases by 8.4% when compared with short and high age of grass regrowth. [48] and [51] confirmed that the level of dry matter intake was negatively correlated to the hemi-cellulose and cellulose (NDF) content. But as described by the low coefficient of correlation, ($r = -0.65$ and $r = -0.31$), NDF alone appears as a bad predictor of intake.

Nutrient Concentrations and Requirements

The nutrients concentrations of forages are factors of interest in the regulation of food intake. On a "requirement theory" basis, the animal eats in order to maximize its production potential under some constraints such as its gut volume and diet quality [52]. According to this theory, intake regulation would be based on the meeting of energy needs [53, 54]. [55] and [56] have demonstrated that intake is positively linked to the body weight and to the level of production of dairy cows and therefore, linked to the animal's requirements. In a meta-analysis performed [51], factors linked to dairy cow needs and performances, such as animal body weight, change in body weight and milk yield, explained 71% of the total variations observed in dry matter intake. [57] confirmed that dry matter intake is strongly correlated to both nutrient digestibility and animal's requirements. As a consequence, ruminants eating very fibrous forages are generally unable to cover their energy needs [48].

In the area of energy needs, animal physiological state appears to be an important factor controlling voluntary intake. As an example, lactating dairy cows with higher energy requirements, graze more selectively and intensively than dry cows [58]. [43] and [44] reported that, there exist a critical rumen fill above which dry matter intake is limited. This critical rumen fill could be differently related to the physiological status of the animal. So, the introduction of ballast into the rumen of dairy cows when the energy requirements are high (beginning of lactation), involves an important decrease of intake level (0.099 to 0.043 kg dry matter per liter of added bulk). To explain energy need, cows in early lactation compensate with increasing the ruminal volume, decreasing the volume of digesta and increasing the passage rate of digesta. If the same experiment is repeated with dairy cows later in their lactation, when their energy requirements are lower, intake does not seem to be modified by the introduction of the ballast in the rumen. The pregnancy state is also a factor controlling the voluntary intake. In the predictive equation [59], intake capacity of dairy cows is proportional to lactation state, age and maturity of the cow, and to a pregnancy indicator that explains the decrease of intake during the last weeks of pregnancy. Animals can also regulate their own intake in relation to forage nutrient concentration. [60] has shown that, if sheep have the choice between two diets with different energy levels, they choose the diet that presents the highest energy density. If sheep have no choice, they adapt their intake until their requirements are met. The energy and protein balance of the diet can also influence the level of intake and the diet selection. So, lambs that select between pairs of diet, varying between 7.8 and 23.5% of crude protein, present a maximal level of intake with the diets containing between 14.1 and 17.2% of crude proteins [61]. Based on these observations, a judicious forage supplementation could contribute to improve diet nutritional balance and also increase the total voluntary intake [62, 63, 51].

Pre - and Post-Ingestive Feedback

The level of intake can be conditioned by other characteristics of the forage, such as flavor (taste and smell), appearance and texture and also by the post-ingestive feedback occurring after its intake. In other terms: "if forage tastes good, animals tend to eat it more" [64]. The flavor – feedback interaction depends

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directly on chemical characteristics of feed, animal's nutritional status and animal's past or recent experiences. According to [65], the training of the animals can be made in various ways. Animals learn from their mothers before and after birth, from pair groups, testing new food and accepting or rejecting them according to the consequences induced by intake. This can explain why the preference is never fixed.

The post-ingestive feedback can evolve in relation to new experiences performed by the animal [66]. Ruminants are able to learn about the toxicity of a feed. For grazing and browsing ruminants, numerous plants contain secondary metabolites like tannins and substances that could affect digestibility, cause negative effects and reduce voluntary feed intake. Ruminants are able to use this information to modulate or avoid intake of such plants, if necessary. For instance, browsing goats tend to reduce their preference for browses sprayed with lithium chloride, chemical components associated with high negative post-ingestive feedback; tannins may reduce grazing of some forage legumes such as *Lotus pedunculatus* by affecting ruminal fermentations [67]; terpenes can inhibit cellulolytic activity of rumen micro-organisms and so limit the intake of such plants.

Finally, regulation of intake appears mediated by different signals, under the form of metabolites and hormones, emitted by the central nervous system and the peripheral organs like liver, pancreas and intestinal tracts that can be regarded as mediators of appetite [39]. The role of leptin, (hormone secreted by fatty cells), cholocystokinine, (hormone secreted by the intestinal mucous membrane), and insulin that controls satiety in ruminants have been widely documented [68, 40]. During digestion process, the rumen environment, (P^H and osmolality) can also explain the variation of voluntary intake [59, 56]. As demonstrated by [60], sheep are able to make short-term changes in diet selection to maintain good ruminal fermentations and wellbeing sensation.

Plant Morphological Characteristics

Sward characteristics, in terms of blade morphology, such as hair occurrence, thickness of cuticle [69], leaf size, stem physical properties, dead materials ratio, can stimulate, limit or inhibit animal foraging behavior [65]. These parameters have huge influence on bite size and intake rate. [70] reported that, in relation to grass characteristics, bite size can vary from 10 to 400 mg for sheep and from 70 to 610 mg for cattle. Under grazing, there is a close relationship between leaf proportion [50, 71], green leaf mass [72, 73], sward density [70] and dry matter intake.

According to [74], stems can have a barrier effect on bite size and instantaneous intake rate. The higher the stems density, the smaller the bite area and the slower the biting rate. This leads to a decrease in the instantaneous intake rate. [75] confirmed that stem length and proportion in the sward have a negative impact on biting rate with correlation of - 0.67 and - 0.40 respectively. Sward composition, in terms of plant species, can also influence the level of intake. Indeed, compared to grasses, legumes such as white clover are often associated to higher level of intake [76]. [64, 38] explained that forage legumes are faster reduced in small particles than grasses and that less time is needed to take and masticate a similar bite of clover than grass.

Role of Environment in Feed Intake

Intake during grazing does not depend only on diet quality. Short-term intake rate is also directly correlated to forage distribution and availability [77]. This explains the lower level of intake observed under tropical rangeland, where forage resources are scattered and/or heterogeneous, which results to reduction in biting frequency and intake rate due to the time spent to go from one favorite site to another [78]. Nevertheless, animals compensate biting rate decrease by increasing grazing time. According to [58], when sward availability is measured through sward height decreases, cows increase their total grazing times, total jaw movement and total number of bites in order to maintain daily intake. These observations are confirmed on tropical forages [75].

Climatic conditions also play an important role. Ruminant animals graze essentially during daylight. In temperate climate, they make 6 to 8 meals with 2 being main meals, one at the beginning and one at the end of the day. If temperature is higher than 25°C, they adapt grazing to early morning hours or late evenings in order to avoid the hot periods of the day and this decreases the time spent grazing and total daily intake [41].

Environment also plays an important role in resource utilization. On one hand, animals have a memory of food allowance, location and distribution as related in the review of [79]. On the other hand, the rearing practices can explain the ability of cows to graze specific environment such as mountain slopes depending on the animal breeds [80]. Interaction between animals in the herd is sometimes cited to explain difference in animal grazing behavior. [81] reports that, on homogeneous vegetation, total time spent grazing by Scottish blackface sheep is higher when space allowance is high (200m2 per head *vs* 50m2 per head) without impact on herbage intake or digestibility. They conclude that the relation between time spent grazing and space allowance can be used to explain the extra activity required to maintain the group cohesion when space allowance increases.

Intake Measurement under Grazing

Direct intake measurement methods are based on herbage mass measurement. In most cases, intake is estimated by the method of difference as used [82].

ISSN: 0975 - 4539
IJBSAT (2013), 5(3):15-25

The method implies the knowledge of herbage mass before and after grazing. The herbage mass is usually estimated by cutting and weighing the grass harvested on a defined area. A sward height meter or rising plate meter or disk meter may also be used to estimate grass density and quantity. The different methods are easy to apply and give reliable results if grazing period is short, (one or two days at the maximum), and stocking rate is high, (ideally all the grass of the grazing area must be consumed). If grazing period is longer, the error of estimation, linked to the grass regrowth during this period, is the major disadvantage of these methods.

To reflect the effect of grass regrowth, herbage mass and regrowth are measured in cages that exclude grazing animals. By successive cuttings, the grazing is simulated and the herbage mass accumulation is measured. But without urinary and dung restitution, specific defoliation linked to the grazing, the measured grass accumulation is often very different in grazed or non-grazed area [83]. The precision of the cutting methods is essentially based on the sampling methodology and a good precision is required at all steps of the protocol to avoid the addition of errors in the measurements. These different techniques are used to measure the intake of animal herds [82]. Instantaneous intake can also be directly estimated through live weight differences [84]. With that method, it is possible to measure intake only over a very short period (1 hour as an example). The accuracy of the measurements is strongly dependent on the precision of balance and weight loss related to dung and urine excretion during the period of measurements.

Another method of intake estimation is based on the hypothesis that the knowledge of animal requirements and performances is a good reflection of the nutritive values of ingested diet. The method is often used to determine the potential of intake of dairy cows as described [56]. Grazing supplemented dairy cows to determine grass intake from animal performances is reliable and less expensive than other methods.

Beef cattle were used to estimate intake from live weight and rate of growth with a good accuracy (residual standard deviation of 8.7% of the mean). The difficulty of the method is precisely the determination of the true herbivore requirements. This is particularly true under tropical rangeland where many external factors like displacements, feed researches must be added to basal animal needs [32].

Intake of grazing ruminants can be estimated by indirect methods such as the markers techniques, ratio techniques, the recording of animal behavior and other empirical models. The markers technique implies the determination of natural indigestible plant components, such as lignin, alkanes, or insoluble ashes which are excreted in feces. The N-alkanes method appears as the best to estimate intake under grazing. The method, based on the determination of the concentration in plant and feces, natural alkanes and synthetic alkanes, allows the researcher to calculate intake from: $I = (Fi/Fj) \times Dj / (Hi - (Fi/Fj) \times Hj)$, where I = intake; Fi and Fj = concentration of natural and synthetic alkanes in feces; Dj = dose rate of synthetic alkanes; Hi and Hj = concentration of natural and synthetic alkanes in forage. Ratio technique implies the determination of two parameters: forage digestibility and fecal output that allows estimating intake: If $D = 100 \times (I - F) / I$, then $I = F / (1-D/100)$ where D = forage digestibility coefficient (%); I = intake (weight unit per day); F = total fecal excretion (weight unit per day) [63].

[85] reported several methods for the determination of fecal output. The total collection of feces is one of the methods, though it is difficult to apply at grazing. To collect feces, animal must be harnessed with fecal bag or tethered, which could interrupt grazing behavior [63]. Quantity of feces that are difficult to estimate could escape from the collection bags and this could be a source of error in the estimation of intake [85]. Another method to estimate fecal output is the use of indigestible external markers as chromium oxide, ytterbium. Total collection of feces using dosing of markers requires a daily manipulation of the animals. The developed controlled release device technique, which pulses automatically a daily amount of external markers in rumen throughout the trial period, allows limiting the animal manipulation and seems sufficiently accurate to give a good estimate of fecal output [86]. Markers technique has difficulty in the sampling of forage that must be representative of the ingested diet. The N-alkanes in plant organs and plant species present different N-alkanes profiles [87] and this is a major source of error in the estimation of intake. If digestibility is determined on a non-representative sample of grazed grass, the estimation of intake will be biased. The hand plucking method, that simulates the biting of the herbivore, may be used to sample grazed grass. The measurement is linked to the calibration between animals and operator's observations. Such calibration appears easier to set up with cattle than with sheep and goats that are more selective in grazing behavior [88].

The use of oesophageal fistulate appears unfavourable to animal welfare and can modify herbivore behavior, as reported in several studies [89, 90]. Intake can be indirectly estimated by studying the grazing behavior. Indeed, intake is the product of three parameters: grazing time, biting rate and bite mass. Grazing time and biting rate can be measured by visual observation [2]. The method is easier to apply and does not require costly equipment. Nevertheless, the presence of the observer can disturb animal grazing, so, it is primordial to accustom animals to the observer in order to avoid

any behavior modification [91]. The recording of animal activities such as displacement, rumination and intake times have been largely tested and used to determine grazing time and biting rate [92]. These recording methodologies require expensive materials and the harnessing of the animal with recording apparatus can disturb its behavior. Such techniques are difficult to apply with wild herbivores and on heterogeneous rangeland.

Another alternative is the micro-histological analysis of plant residue contents in feces or the stomach and intestinal tract. The method is often used to approach the intake of wild ruminants. The main disadvantages of this technique are that, except for feces collection, it requires the slaughter of the animal and that the identification of the ingested plant fraction, till the species level, is very difficult due to the digestion process [93].

Moreover, the quantitative estimation of the different ingested plant fractions are very few as the quantity of plant fragments found in feces or in stomachs are not, due to differential digestibility, directly proportional to the quantity of the ingested plant fractions.
During the last 30 years, many empirical models have been developed to estimate forage intake at pasture. These models are based on multiple regressions between intake level and some characteristics of plant (OM yield, fiber content, digestibility, part of legumes, etc.), animal (live weight, average daily gain, milk production, stage of lactation, milk composition, pregnancy, etc.) and environment (temperature, rainfall, etc.). Most of these models derive from dairy cows experimental data [94]. They are often specific to the relatively short range of grazing and experimental conditions used to develop them.

CONCLUSION

Since evaluation of preferred forage will continue to be important in grazing lands development, it is necessary to spatially separate the forages being evaluated to eliminate the constraints that occur within an intimately mixed sward. This is most critical for researchers in regions which are trying to move from traditional extensive pastoral practices to some form of semi-sedentary production of ruminants under a managed field. As these reviewed novel approaches continue to evolve, it is expected that they will become simplified and cost effective and, therefore, find wider applications in agriculture and diagnostic research.

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